

This article was downloaded by:[Universiteit Gent]
[Universiteit Gent]

On: 21 March 2007

Access Details: [subscription number 769486293]

Publisher: Informa Healthcare

Informa Ltd Registered in England and Wales Registered Number: 1072954

Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Growth Factors

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t713642964>

Platelet-rich plasma preparation using three devices: Implications for platelet activation and platelet growth factor release

Peter AM Everts^a; Christine Brown Mahoney^b; Johannes JML Hoffmann^c; Jacques PAM Schönberger^d; Henk AM Box^a; André van Zundert^e; Johannes TA Knap^f

^a Department of Extra Corporeal Blood Management, Catharina Hospital Eindhoven. Eindhoven. The Netherlands

^b Business College and Statistics, Winona State University. Winona, MS. USA

^c Department of General Laboratories, Catharina Hospital Eindhoven. Eindhoven. The Netherlands

^d Department of Cardiothoracic Surgery, Catharina Hospital Eindhoven. Eindhoven. The Netherlands

^e Department of Anaesthesiology, Catharina Hospital Eindhoven. Eindhoven. The Netherlands

^f Department of Anaesthesiology, University Medical Centre. Utrecht. The Netherlands

To link to this article: DOI: 10.1080/08977190600821327

URL: <http://dx.doi.org/10.1080/08977190600821327>

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

© Taylor and Francis 2007

Platelet-rich plasma preparation using three devices: Implications for platelet activation and platelet growth factor release

PETER AM EVERTS¹, CHRISTINE BROWN MAHONEY³, JOHANNES JML HOFFMANN², JACQUES PAM SCHÖNBERGER⁴, HENK AM BOX¹, ANDRÉ VAN ZUNDERT⁵, & JOHANNES TA KNAPE⁶

¹Department of Extra Corporeal Blood Management, Catharina Hospital Eindhoven, Eindhoven, The Netherlands,

²Department of General Laboratories, Catharina Hospital Eindhoven, Eindhoven, The Netherlands, ³Business College and Statistics, Winona State University, Winona, MS, USA, ⁴Department of Cardiothoracic Surgery, Catharina Hospital Eindhoven, Eindhoven, The Netherlands, ⁵Department of Anaesthesiology, Catharina Hospital Eindhoven, Eindhoven, The Netherlands, and ⁶Department of Anaesthesiology, University Medical Centre, Utrecht, The Netherlands

Abstract

Background: In this study, three commercial systems for the preparation of platelet-rich plasma (PRP) were compared and platelet growth factors release was measured.

Methods: Ten healthy volunteers donated whole blood that was fractionated by a blood cell separator, and a table-top centrifuge to prepare PRP. Furthermore, an autologous growth factor filter was used to concentrate PRP fractionated by the blood cell separator. PRP was subsequently activated with autologously produced thrombin to degranulate the platelets to measure platelet-derived growth factor-AB (PDGF-AB), transforming growth factor-beta (TGF- β), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF).

Results: PRP contained significantly higher platelet counts compared with baseline values ($p < 0.001$). PDGF-AB concentrations were increased more than 18-fold in the platelet gel supernatant when the cell-separator and GPS were used, whereas only a 3-fold increase was seen with the AGF.

Conclusion: The three PRP devices enable the preparation of PRP for the release of high concentrations of platelet growth factor, but showed different harvesting capacities for the collection of concentrated platelets. The administration of thrombin for PRP activation resulted in the release of high concentrations of PDGF-AB and TGF- β but only when PRP had not been activated during the preparation process *in vitro*.

Keywords: Platelet gel, platelet growth factor, platelet-rich plasma, platelet activation

Introduction

Traditionally, platelets are used therapeutically to correct thrombocytopenia or platelet dysfunction (Sensebe et al. 2005). It is currently understood that platelets also play a pivotal role in the repair of injured tissues since they contain platelet growth factors that is released from activated platelets; platelet growth factors initiate and modulate wound healing in bone or soft tissue (Bourquie et al. 1993, Mazzucco et al. 2004). Of particular importance in these healing

processes is platelet-derived growth factor (PDGF), which exists as both heterodimers of A and B chains and as homodimers of either A–A or B–B chains (Pierce et al. 1991). Also of importance are transforming growth factor-beta (TGF- β) (Rosier et al. 1998), vascular endothelial growth factor (VEGF) (Rhee et al. 2004), smaller amounts of insulin-like growth factor (IGF) (Weibrich et al. 2002a) and epidermal growth factor (EGF) (Martin et al. 1992). Additionally, Kubota and others described that connective tissue

Correspondence: Peter AM Everts, Department of ECC and Blood Management, Catharina Hospital, Michelangelolaan 2, 5623 EJ, Eindhoven, The Netherlands. Tel.: 31 40 2399111. Fax: 31 40 2912480. E-mail: everts@elive.nl

growth factor (CTGF) is also present (Kubota et al. 2004). Recently, strategies in clinical treatment plans encourage the production of autologous platelet-rich plasma (PRP) containing high concentrations of platelet growth factors with the use of whole blood separation devices. PRP mixed with thrombin and calcium chloride will result in the production of platelet gel, which can be exogenously applied to surgical wounds, leading to the degranulation of the platelet α granules and platelet growth factor release (Fr  chette et al. 2005). In several studies, investigators have appeared to take advantage of platelet growth factor delivery in support of hemostasis and wound healing (Englert et al. 2005, Everts et al. 2006). In oral and maxillofacial surgery, published results imply that earlier bone graft maturation can be expected when platelet gel is used in mandibular defects (Marx et al. 1998, Steigmann and Garg 2005). In addition, platelet gel applications have also been reported to improve soft tissue healing in chronic non-healing wounds (Margolis et al. 2001, Crovetti et al. 2004). Before clinicians began to routinely use platelet gel, it was logical to analyze the manufacturing procedure for PRP, which may result in varying amounts, quality, and efficacy from patient to patient, and to characterize the platelet growth factor content. The purpose of this study was to evaluate the PRP preparation method in 10 healthy volunteers and compare the results obtained using three different commercially available devices with different operating principles. The specific objectives were: 1. To compare platelet counts, 2. To determine whether platelet activation occurs during the PRP preparation process, 3. To examine the effects of PRP storage on growth factor levels, and 4. To quantitate certain platelet growth factors (i.e., PDGF, TGF- β , VEGF, and IGF) in the platelet gel.

Material and methods

Study design

The study was approved by the Institutional Ethics Committee of the Catharina Hospital (Eindhoven, The Netherlands) (World Medical Association 2002). Written, informed consent was obtained according to the guidelines. Each volunteer was identified by number, enabling a reviewer to identify and compare the test results. The study was consecutively executed in five working days.

Materials

The Gravitational Platelet Sequestration SystemTM, (Biomet Co, Warsaw, IN, USA) (GPS), is a table-top centrifuge system using a flat-bottom, 60-ml plastic centrifuge tube, containing a buoy and an internal coiled device located in the tube cap. The buoy is

lowered to remove the platelet-poor plasma following a 12 min spin at 3200 rpm. Thereafter, the PRP volume (± 5 ml) can be collected. With this device, the red blood cells cannot be collected separately and are therefore discarded.

The Electa Cell-SeparatorTM (Sorin Group, Mirandola, Italy) (CS) is an intraoperative blood salvage device that uses a Latham bowl to separate the PRP from the whole blood and utilizes modified software for apheresis (11). One PRP production procedure incorporates both a 5400-rpm hard spin and a 2400-rpm soft spin to collect the PRP. The platelet poor plasma and erythrocytes are collected separately in two transfusion bags and retransfused to the patient. The PRP is collected in a 60-ml syringe.

The Autologous Growth Factor FilterTM, (Interpore Cross[®], Irvine CA, USA) (AGF) is a microporous, hollow, cellulose fiber filtration device with a volume of 8 ml and uses PRP as a baseline product that has been manufactured by the cell separator device. A specific manifold is needed for the AGF, which has two 60-ml syringes attached that are driven by compressed air. The device filters water after multiple passes of PRP through the filtration device. The end-product is concentrated PRP. All three devices were used according to the manufacturer's instructions.

Methods

PRP preparation

An intravenous infusion line was inserted into the medial cubital vein using a 17-g needle. For each donor, whole blood donation was distributed as 2 aliquots. First, a 60-ml syringe was pre-filled with 7 ml of anticoagulant citrate dextrose A solution and 53 ml of whole blood was slowly drawn via an intravenous catheter. The syringe was inverted five times to ensure proper mixing with the anti-coagulant before PRP was prepared using the GPS. An additional 375 ml of blood was passively drained into a blood bag containing 53 ml of citrate-phosphate-adenine anticoagulant-preservative to produce PRP using the CS, which was programmed to perform platelet sequestration. The PRP from the CS was divided into 3 aliquots: 4 ml for the preparation of autologous thrombin, 3 ml was kept for platelet gel fractionation, and the remainder was used for the concentration of PRP using AGF. The PRP prepared using the GPS was solely used to produce platelet gel.

Platelet gel enrichment

Following PRP preparation, autologous thrombin was isolated to activate the PRP and thus to prepare platelet gel. Autologous thrombin was isolated from 4 ml PRP and 0.17 ml of 10% calcium chloride to antagonize the anticoagulant present in the donated

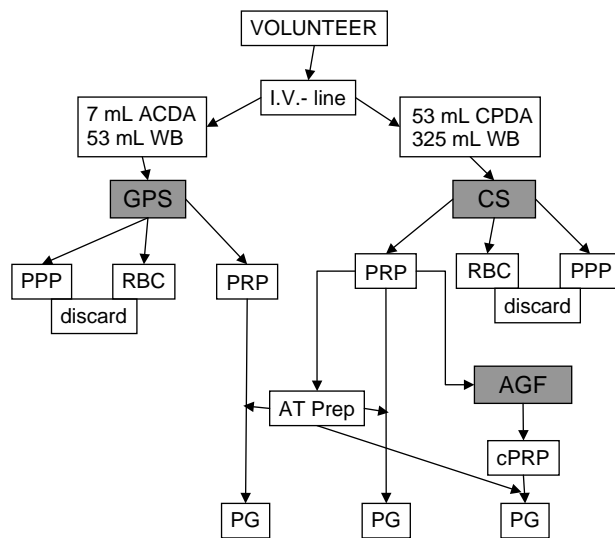


Figure 1. I.V.-line: intra venous line; ACDA: anticoagulant citrate dextrose A CPDA: citrate-phosphate-adenine anticoagulant-preservative; WB: whole blood; GPS: GPS table top; CS: Electa cell-saver; PPP: platelet-poor plasma; PRP: platelet-rich plasma; RBC: red blood cells; AT Prep: autologous thrombin preparation; AGF: autologous growth factor filter; cPRP: concentrated platelet-rich plasma; PG: platelet gel.

blood. This mixture was injected into a glass Petri dish to activate platelets and form a clot. After 25 to 40 min, a clot was formed inside the glass dish and compressed manually. The cellular clot debris was left behind on the dish and the liquid fraction containing the thrombin was aspirated with a syringe. Platelet gel was prepared by mixing PRP and thrombin in a ratio of 4:1. Figure 1 shows a diagram on the study set-up.

Sampling and hematological analysis

Blood samples used for baseline measurements were drawn from the intravenous line of the patient and collected in citrate-phosphate-adenine monovettes. After whole blood centrifugation procedures using the three devices, a sample of the PRP volume was taken for laboratory analysis. Subsequently, the PRP of the GPS and 3 ml of the CS were placed onto a flatbed agitator at a frequency of 60 rpm and stored at room temperature ($21 \pm 2^\circ\text{C}$) for 75 min during PRP was concentrated using the AGF. After this period, blood samples were taken from the PRP and concentrated PRP. Then, 3 ml of PRP from the GPS, CS, and concentrated PRP were activated by autologous thrombin to form the viscous coagulum, platelet gel. The platelet gel coagula were placed into three different cups that represented each device; after 75 min, a sample was taken from each supernatant for analysis.

In all blood samples, platelet counts were assessed using a fully automated analyzer (Cell-DynTM 4000, Abbott Diagnostics, Santa Clara, CA, USA) and the impedance method.

Determination of platelet activation

During the entire PRP and platelet gel preparation procedures, platelet activation was determined by measuring plasma levels of beta-thromboglobulin using Asserachrome beta-thromboglobulin kits (Stago, Asnieres, France).

Determination of growth factor concentrations

Platelet growth factor concentrations in whole blood, PRP, concentrated PRP, and platelet gel supernatants were determined by commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis MN, USA) that had been validated for measuring PDGF-AB (Quantikine PDGF-AB), TGF- β 1 (Quantikine TGF- β 1), and VEGF (Quantikine human ANG), according to the manufacturer's instructions. The enzyme-linked immunosorbent assay procedures were programmed into an automated analyzer (Coda Automated EIATM analyser; Bio-Rad Laboratories, Hercules CA, USA). Samples were measured in duplicate and in appropriate dilutions to fit the respective calibration curves. Repeat analysis was performed when between duplicate differences greater than 10% were found. Insulin-like growth factor-1 (IGF-1) was measured using a fully automated chemiluminescence immunoassay in the Advantage Analyzer (Nichols Institute Diagnostics, San Clemente CA, USA).

Statistical method

The study data were gathered on customized patient tracking forms and entered into a computerized database that allowed unbiased and reliable data management. Statistical analysis utilized SAS statistical software (SAS Institute, Cary, NC; USA, 2003). Measured data were expressed as the mean \pm 1 standard deviation (SD). The measurements of interest were the changes within each individual from one time point to the next and the average. The paired *t*-test focused on the difference between the paired data and reported the probability that the actual mean difference was equal to zero. Differences were calculated for all measurements from Time 1 to Time 0, Time 2 to Time 0, and Time 3 to Time 0 for each individual. These differences were averaged for the specific device type. T-tests for significant differences between devices were then calculated, where $p < 0.05$ was considered significant (Time 0 = baseline, Time 1 = after PRP preparation, Time 3 = after 75 min, and Time 4 = after activation by autologous thrombin).

The platelet yield, a measurement to validate the collection efficiency of the devices, was determined using the following formula:

Table I. Characteristics of PRP production[§].

System	WB PLT concentration ($\times 10^3/\mu\text{l}$)	PRP PLT ($\times 10^3/\mu\text{l}$)	PLT increase baseline	PLT yield (%)
CS	219 \pm 81	1051 \pm 276*	4.9 \pm 0.7	48 \pm 12*
GPS	219 \pm 81	569 \pm 247*	2.8 \pm 1.1	36 \pm 15
AGF	1051 \pm 276	1222 \pm 326 [†]	1.2 \pm 0.1	32 \pm 11

[§]All values represent the mean \pm SD (PRP: platelet-rich plasma).

Platelet increase Baseline is a value representing the increase in PRP platelet count compared to the baseline platelet count. PLT: platelet.

* $p < 0.001$, [†] $p < 0.05$. WB: whole blood.

Platelet yield (%) = (PRP volume) \times (platelet count in PRP)/(WB volume) \times (platelet in WB) $\times 100\%$. (WB: whole blood).

Results

Preparation of platelets

Blood samples were collected from 10 healthy volunteers (5 men, 5 women), aged 24 to 54 years (38 ± 9 years, mean \pm SD), who were not taking any medications. All donors in this study had a platelet count greater than $150,000/\mu\text{l}$, and the mean hematocrit was $42 \pm 4\%$ (SD). In Table I, the characteristics of the PRP preparation for the three devices are compared. With each device, we found that platelet numbers significantly increased compared with the baseline donor platelet count for the CS, GPS ($p < 0.001$), and AGF ($p < 0.05$). After comparing the mean PRP platelet counts, it was noted that PRP prepared using the CS contained significantly more platelets ($p < 0.001$) than preparations using the other two devices. Furthermore, the CS resulted in a significantly higher platelet yield than those obtained using the GPS and AGF: 47%, 36%, and 32%, respectively. The relative increase in platelet count above baseline platelet count in the whole blood sample was the lowest for the AGF, despite the fact that the PRP volume was reduced 2.5-fold with the AGF.

Platelet activation during PRP preparation

Activated platelets were shown to release beta-thromboglobulin; these results are shown in Table II.

The beta-thromboglobulin levels for both the CS and GPS were not statistically different following PRP preparation. After PRP activation by autologous thrombin, release was 10-fold higher than in non-activated PRP ($p < 0.001$ for both systems). PRP storage had no significant effect on platelet activation. In contrast with the CS and GPS, we noted a significantly higher beta-thromboglobulin concentration after filtration procedure in the concentrated PRP ($p < 0.001$) using the AGF. When the concentrated PRP was activated by thrombin, only a minor release could be measured, indicating less residual platelet activation.

Growth factor release into the PRP supernatant

In Table III, the IGF-1 and VEGF data are shown. Low levels of IGF-1 were detected in the supernatants from non-activated PRP and did not significantly increase after the addition of thrombin with any of the three devices. Mean VEGF concentrations were detectable in the PRP of the CS and GPS (204 ± 53 and 191 ± 36 ng/ml, respectively) and in the concentrated PRP of the AGF (250 ± 80 ng/ml).

The results in Table IV 1 reflect the PDGF-AB and TGF- $\beta 1$ levels after PRP, concentrated PRP preparation, and subsequent platelet gel production. Compared to the CS and GPS, we observed significantly increased levels of both PDGF-AB and TGF- β immediately after concentrated PRP preparation using AGF ($p < 0.001$). After activation by thrombin, the PRP of the CS and GPS resulted in a significant increase in growth factor release ($p < 0.001$) when compared with the AGF. The largest increase was observed by the release of

Table II. β -TG activation (IU/ml) during PRP separation[§].

System	PRP		After 75 min storage PRP
	Non-activated	Thrombin-activated	
CS	1666 \pm 1058	24,207 \pm 13,117*	2420 \pm 1443
GPS	1741 \pm 1417	23,130 \pm 10,820*	2164 \pm 1407
AGF [†]	26,574 \pm 10,185*	38,629 \pm 14,732	N/A

[§]All values represent the mean \pm SD.

cPRP: concentrated platelet-rich plasma; PRP: platelet-rich plasma, N/A: not applicable.

* AGF product is concentrated PRP, [†] $p < 0.001$.

Table III. IGF and VEGF concentrations after PRP separation and thrombin activation[§].

System	IGF-1 PRP ($\mu\text{g/l}$)		VEGF PRP (ng/l)	
	Non-activated	Thrombin-activated	Non-activated	Thrombin-activated
CS	100 \pm 29	87 \pm 34	204 \pm 53	144 \pm 49
GPS	99 \pm 29	107 \pm 29	191 \pm 36	191 \pm 37
AGF*	132 \pm 32	102 \pm 35	250 \pm 80	219 \pm 58

[§]All values represent the mean \pm SD.

PRP: platelet-rich plasma.

* AGF product is concentrated PRP.

PDGF-AB (more than 18-fold compared with the PRP levels).

Discussion

In this study, we observed that the PRP devices particularly differ in their abilities to collect concentrated and non-activated platelets from whole blood for the enrichment of PRP. High concentrations of PDGF-AB and TGF- β were released after PRP activation by autologous thrombin.

Under normal circumstances, wound healing is initiated through platelet activation and aggregation in the presence of calcium and thrombin, generating a fibrin clot in which a matrix for migration of tissue-forming cells will be formed (Lin et al. 1997). During the wound-healing cascade, platelets are trapped and activated, resulting in the release of growth factors present in the α -granules of the platelets (Slater et al. 1995). The effects of autologous concentrated platelet growth factors, delivered as a platelet gel, have been studied to some extent during various surgical procedures (Marx et al. 1998, Margolis et al. 2001, Crovetto et al. 2004, Englert et al. 2005, Steigmann and Garg 2005, Everts et al. 2006). At present, no standardized protocols for peri-operative harvesting of PRP and subsequent platelet gel application have been formulated, although the number of PRP-producing devices is increasing. The currently available PRP processing devices have rarely been validated with ample information for the clinician concerning the quality of the manufactured PRP and the growth

factor content of the platelet gel (Kevy and Jacobson 2004, Weibrich et al. 2005). Zimmerman and co-workers discussed this lack of standardized protocols. They applied standard blood bank criteria to investigate different methods for the preparation of platelets and identified factors that influence the production of platelet gel (Zimmerman et al. 2001). It was our objective to compare platelet collection efficiency, monitor platelet activation during PRP preparation, and observe platelet growth factor release after PRP activation using three different commercial point-of-care devices. The technical design and operating principles of the devices that produce PRP are completely different, but are all presently being used in clinical settings. The use of the CS demands larger whole blood volumes to prepare PRP. We routinely use this device either in combination with red cell salvage techniques, or when larger platelet gel volumes are therapeutically indicated. The advantage of this device is that the disposable kit can be used repetitively to sequester whole blood. Furthermore, all produced blood components are retransfused to the patient using aseptic techniques to avoid contamination. The GPS is a low-volume device and prepares therefore small PRP volumes. The disposable set can be used once. Due to the construction of the GPS blood separation chamber, the manufacturer recommends sterile gloves be worn during the PRP collection steps, which is performed manually. The AGF is a modified hemoconcentrator that requires PRP be prepared using a cell salvage device for further PRP concentration. Nevertheless, in some papers, the

Table IV. PDGF-AB and TGF- β 1 values after PRP separation and thrombin activation[§].

System	PDGF-AB (pg)		TGF- β 1 (pg)	
	Non-activated	Thrombin activated	Non-activated	Thrombin-activated
CS	8982 \pm 1899	145,995 \pm 61,259*	2501 \pm 899	8798 \pm 2301*
GPS	4194 \pm 1009	109,908 \pm 48,885*	1379 \pm 785	4102 \pm 1734 [‡]
AGF [†]	151,766 \pm 47,989*	119,747 \pm 48,723	5389 \pm 1452*	3285 \pm 1288

[§]All values represent the mean.

PRP: platelet-rich plasma. PG: platelet gel.

* AGF product is concentrated PRP.

[‡] $p < 0.001$, [†] $p < 0.05$.

effect of applying concentrated PRP to tissues to increase growth factor delivery revealed no effect (Lowery et al. 1999, Weiner and Walker 2003, Castro 2004, Carreon et al. 2005). To account for the device differences in preparation volume, and therefore PRP volume, we calculated the total amount of growth factors rather than measuring the individual concentrations of PDGF-AB and TGF- β .

The results of our investigation are to some extent in accordance with the data of Kevy and Jacobson (Kevy and Jacobson 2004). We showed that the overall platelet collection efficiency and platelet count of the PRP were dissimilar for the three devices. The cell-salvage device significantly showed the highest platelet recovery (platelet yield of 48%) and the highest platelet count in the PRP than the other two devices. The lowest platelet yield was measured in the AGF device. Despite the fact that the actual PRP volume of the AGF system was decreased by a factor of 2.8, we did not observe an equal rise in platelet counts, which means that platelets were lost during the concentration process. Weibrich et al. performed similar studies using the PCCSTM system and the Anitua Platelet-Rich-in-Growth Factor Kit (Weibrich et al. 2005). They also demonstrated significantly different platelet collection efficiencies and platelet counts, supporting our impression that there might be a large variation among devices for the harvest of PRP.

In our study, we observed that the different platelet harvesting methods had a significant effect on the activation of PRP and platelet growth factor release from the platelet gel. Therefore, it is critical to validate and understand the PRP preparation process before platelet gel techniques are applied to patients. To evaluate the quality of the PRP preparation process, we monitored platelet activation. An established *in vivo* method to measure platelet activation is the detection of β -TG as a marker of platelet activation. The AGF system showed the highest β -TG release after PRP processing. In our opinion, this finding might be the result of the repetitive passes of the platelets through the microporous fibers of the hemoconcentrator. The platelets adhere to the surface of the hollow fibers, a phenomenon that is well described with the use of these devices during dialysis (deSa et al. 2001). This is the most likely reason why the increase in platelet count of the concentrated PRP was not in accordance with our expected 2.8-fold increase in platelet count. In contrast to this finding, we noticed that PRP obtained with the other two systems demonstrated minimal platelet activation, even after a 75-min storage period. When PRP was activated by thrombin, platelet gel was formed and platelets were immediately activated as measured by beta-thromboglobulin levels that increased 12.3- and 13.5-fold compared with the PRP levels for the GPS and CS devices, respectively. The concentrated PRP of the AGF showed only a 3.2-fold increase in platelet activation, indicating that

the vast majority of platelets in the concentrated PRP had been activated before platelet gel was prepared.

During clinical platelet gel applications, concentrated and activated platelets are exogenously applied to wound sites where they adhere to tissues and start to degranulate, resulting in the release of platelet growth factors. Slater et al. and Spencer and co-workers have demonstrated that platelet growth factors have mitogenic and chemotactic properties that may contribute to improved wound healing (Spencer et al. 1993, Slater et al. 1995). Therefore, it is important that platelet gel applied *in vivo* can deliver viable growth factors. This ability decreases when PRP becomes activated *in vitro* before platelet gel is delivered to tissues. For this reason, we measured platelet growth factor concentrations after the preparation of PRP and concentrated PRP, and after activation by autologous thrombin. Compared to the levels of growth factors found with the use of the CS and GPS systems, we observed a significant increase in PDGF-AB and TGF- β concentrations in the concentrated PRP samples, strongly suggesting premature growth factor release prior to thrombin activation with the concentrated PRP method. Therefore, less PDGF-AB and TGF- β was measured in the platelet gel supernatant when compared to the other two devices. The AGF end product is therefore not a viable platelet concentrate but rather a platelet releasate. After activation by autologous thrombin, a significant increase in PDGF-AB and TGF- β levels was measured in the platelet gel supernatants of both systems, whereas the platelet gel in accordance with the results of our β -TG measurements, indicated massive platelet activation following the use of the AGF system and only a minor response to autologous thrombin activation. As demonstrated by Weibrich et al, the IGF-1 concentrations in PRP as well as in platelet gel were not statistically different and remained very low (Weibrich et al. 2002b). An explanation for this observation might be that IGF-1 released from platelets is very low since the plasma pool of IGF-1 is greater than the platelet pool (Karey et al. 1989) and that IGF-1 is primarily excreted by the liver into the plasma (Rubin and Baserga 1995). The mean VEGF values after activation by thrombin were almost similar in PRP and concentrated PRP levels, suggesting that activation by autologous thrombin and calcium does not significantly increase VEGF release during the incubation period.

In conclusion, we studied three different methods to obtain PRP for the *in vitro* production of platelet gel. Our findings indicate that PRP can be sequestered and concentrated 5-fold from one unit of whole blood. Moreover, a comparison of the devices demonstrated discrepancies in both quality of the PRP preparation method and quantity of platelet growth factors released after thrombin activation. With the CS and GPS systems, we could reproduce consistent data

regarding growth factor release, specifically for PDGF-AB and TGF- β . However, the AGF system appeared to generate concentrated, but activated, platelets that released growth factors prior to the preparation of platelet gel. Although our study was not directed toward measuring biological effects of platelet gel, it is likely that the clinical effectiveness of platelet gel prepared with the AGF device is lower than those of the two other systems. Acceptance of standardized protocols for PRP enrichment and subsequent platelet gel application should be instituted in order to achieve and maintain a high quality biological product. Further research is mandatory to assess the healing applications of platelet gel and to determine its effects in a variety of medical disciplines.

Acknowledgements

The authors thank Ms Maria van de Bos and Ms Mary Machin for their assistance during the preparation and determination of the laboratory samples and the volunteers for their participation in the study.

References

- Bourquie WT, Gross M, Hall BK. 1993. Expression of four growth factors during fracture repair. *Int J Dev Biol* 37:573–579.
- Carreon LY, Glassman SD, Anekstein Y, Puno RM. 2005. Platelet gel (AGF) fails to increase fusion rates in instrumented posterolateral fusions. *Spine* 30:243–247.
- Castro FP Jr. 2004. Role of activated growth in lumbar spinal fusions. *J Spinal Disord Tech* 17:380–384.
- Crovetti G, Martinelli G, Issi M, et al. 2004. Platelet gel for healing cutaneous chronic wounds. *Transfus Apher Sci* 30:145–151.
- Englert SJ, Estep TH, Ellis-Stoll CC. 2005. Autologous platelet gel applications during cardiovascular surgery: Effect on wound healing. *J Extra Corpor Technol* 37:148–152.
- Everts PAM, Devilee RJJ, Brown-Mahoney Ch, et al. 2006. Platelet gel and fibrin sealant reduce allogenic blood transfusions and in total knee arthroplasty. *Acta Anaesth Scand* 50:593–599.
- Fréchette J-P, Martineau I, Gagnon G. 2005. Platelet rich plasmas: Growth factor content and roles in wound healing. *J Dent Res* 84(5):434–439.
- Karey KP, Sirbasku DA. 1989. Human platelet-derived mitogens. II. Subcellular localization of insulin like growth factor I to the alpha-granule and release in response to thrombin. *Blood* 74:1093–1100.
- Kevy SV, Jacobson MS. 2004. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol* 36(1):28–35.
- Kubota S, Kawata K, Yanagita T, Doi H, Kitoh T, Takigawa M. 2004. Abundant retention and release of connective tissue growth factor (CTGF/CCN2) by platelets. *J Biochem (Tokyo)* Sep 136(3):279–282.
- Lin PH, Kirko MK, von Fraunhofer JA, Greisler HP. 1997. Wound healing and inflammatory response to biomaterials. In: Chu CC, von Fraunhofer JA, Greisler HP, editors. *Wound healing Closure and devices*. Boca Raton, Fla: CRC Press. p 7–24.
- Lowery GL, Kulkarni S, Pennisi AE. 1999. Use of autologous growth factors in lumbar spinal fusion. *Bone* 25 (Suppl): S46–S50.
- Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. 2001. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 24:483–488.
- Martin P, Hopkinson-Woolley J, McCluskey J. 1992. Growth factors and cutaneous wound repair. *Prog Growth Factor Res* 4:25–44.
- Marx RE, Carlson ER, Eichstaedt RM, et al. 1998. Platelet-rich-plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 85:638–646.
- Mazzucco L, Medici D, Serra M, et al. 2004. The use of autologous platelet gel to treat difficult-to-heal wounds: A pilot study. *Transfusion* 44:1013–1018.
- Pierce GF, Mustoe TA, Altrick BW, Deuel TF, Thomason A. 1991. Role of platelet-derived growth factor in wound healing. *J Cell Biochem* 45:319–326.
- Rhee JS, Black M, Schubert U, Fischer S, Morgenstern E, Hammes HP, Preissner KT. 2004. The functional role of blood platelet components in angiogenesis. *Thromb Haemost* 92:394–402.
- Rosier RN, O'Keefe RJ, Hicks DG. 1998. The potential role of transforming growth factor beta in fracture healing. *Clin Orthop* 355 (Suppl):S294–S300.
- Rubin R, Baserga R. 1995. Biology of disease: Insulin-like growth factor receptor, its role in cell proliferation, apoptosis, and tumorigenicity. *Lab Invest* 73:311–331.
- deSa HM, Freitas LA, Alves VC, Garcao MF, Rosa MA, Marques AA. 2001. Leukocyte, platelet and endothelial activation in patients with acute renal failure treated by intermittent hemodialysis. *Am J Nephrol* 21:264–273.
- Sensebe L, Giraudeau B, Bardiaux L, et al. 2005. The efficiency of transfusing high doses of platelets in hematologic patients with thrombocytopenia: Results of a prospective, randomized, open, blinded end point (PROBE) study. *Blood* 105(2):862–864.
- Slater M, Patava J, Kingham K, Mason RS. 1995. Involvement of platelets in stimulating osteogenic activity. *J Orthop Res* 13:655–663.
- Spencer EM, Tokunaga A, Hunt KT. 1993. Insulin-like growth factor binding protein-3 is present in the α -granules of platelets. *Endocrinology* 132:996–1001.
- Steigmann M, Garg AK. 2005. A comparative study of bilateral sinus lifts performed with platelet-rich plasma alone versus alloplastic graft material reconstituted with blood. *Implant Dent* 14:261–266.
- Weibrich G, Kleis WKG, Hafner G. 2002. Growth factor levels in the platelet-rich plasma produced by 2 different methods: Curasan-type PRP kit versus PCCS PRP system. *Int J Oral Maxillofac Impl* 17:184–190.
- Weibrich G, Kleis WKG, Hafner G, Hitzler WE. 2002. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and PLATELET count. *J Craniomaxillofac Surg* 30(2):97–102.
- Weibrich G, Kleis WKG, Hitzler WE, Hafner G. 2005. Comparison of the platelet concentrate collection system with the plasma-rich-in-growth-factors kit to produce platelet-rich plasma: A technical report. *Int J Oral Maxillofac Impl* 20:118–123.
- Weiner BK, Walker M. 2003. Efficacy of autologous growth factors in lumbar intertransverse fusions. *Spine* 28:1968–1971.
- World Medical Association. 2002. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Nurs Ethics* 9(1):105–109.
- Zimmerman R, Jakubietz R, Jakubietz M, et al. 2001. Different preparation methods to obtain platelet components as a source of growth factors for local application. *Transfusion* 41:1217–1224.